

# SPERMATOGENESIS IN BLASIA

LESTER W. SHARP

WITH PLATE XV

## Introduction

The following brief account of spermatogenesis in *Blasia pusilla* is based upon preparations made from a limited amount of material collected near Chicago several years ago. The preparations, which were originally made for use in classes, proved upon careful examination to show with admirable clearness all stages included in the last spermatogenous mitosis and the transformation of the androcyte (spermatid) into the spermatozoid. Since the results of the examination differ in two important points from those reported by WOODBURN (12) in the only previous paper dealing with these features in *Blasia*, they are here recorded.

## Description

The description will begin with the spermatogenous cells of the penultimate generation, the androcyte mother cells, to use the terminology of ALLEN (1). The cells of the earlier generations (androgones) have been examined, and nothing which it is safe to call centrosomes has been observed. Unfortunately, however, the material did not show many androgone nuclei in division; anaphases were present, but metaphases, where centrosomes are usually most conspicuous if present at all, were not found. No conclusive statement can be made, therefore, regarding the presence or absence of centrosomes in the androgones.

In the androcyte mother cell, before the stage represented in fig. 1, the cytoplasm has an almost homogeneous appearance, and included in it are several granules or vaguely defined areas. In some cells these granules, from 1 to 6 or more in a thin section, may appear to be all alike; while in other cells one or two of them may be more sharply defined and more deeply stained than the others. It is possible that of these several granules two survive as the

centrosomes shown in fig. 1, after the manner of the "black granules" in the body cell of *Dioon* (CHAMBERLAIN 4). On the other hand, it would be possible to select a series of cells illustrating the divergence of daughter centrosomes arising by the division of one, as in *Equisetum* (SHARP 8); or even to show the origin of the bodies in question from the nucleus, as described by WILSON (10) for *Atrichum* and *Mnium*. The writer, however, believes that the evidence afforded by his material is insufficient to support any of these hypotheses in the case of *Blasia*. The present description, therefore, will begin with a stage (fig. 1) at which the identity of the centrosomes is unmistakable, the question of their origin and earlier history being left an open one.

Two centrosomes, whatever may be their previous relation to other cell granules, soon stand out with great distinctness as intensely staining bodies near the cell membrane at opposite poles of the androcyte mother cell (fig. 1). At this time the cell is still rather square in section, since it has only begun to round off from its neighbors, and the centrosomes commonly occupy the corners, as shown in the figure. From each centrosome a conical group of very faint fibers extends toward the nucleus, which is somewhat flattened on the sides facing the centrosomes. While the nucleus is undergoing the prophasic changes (fig. 2) these fibers become more plainly visible, and when the nuclear membrane disappears they become attached to the chromosomes and establish the achromatic figure with the centrosomes at its poles.

It is at metaphase that the spindle is seen most clearly (fig. 3). As noted by WOODBURN (12), it may lie either straight or obliquely in the cell. Furthermore, the cells may round up and alter considerably in shape while mitosis is in progress, so that although the centrosomes may at first be situated near the corners of the cell, all appearance of the diagonal division so characteristic of many bryophytes may in many cases be lost by the time the metaphase and succeeding stages are reached (figs. 4, 5).

When the chromosomes reach the poles at the end of the anaphase (fig. 4), they usually come in contact with the centrosomes. As a result the latter, which are very minute, are often difficult to find at this stage. Careful search, however, reveals cells in which

they stand out clearly a little apart from the chromosome groups. From this time onward they become increasingly distinct. As the membranes form about the reorganizing daughter nuclei at telophase the centrosomes are left just outside in the cytoplasm (fig. 5), and while cell division is being completed they move away from the nucleus and take up positions nearer the cell membrane (fig. 6).

The two androcytes (spermatids), between which no cell wall is laid down, quickly round off from each other (fig. 7). In probably the majority of cases they are somewhat triangular in shape, owing to the usual diagonal plane of the division which differentiates them. In each androcyte the blepharoplast, as we may call the centrosome in view of the function it performs in the cell which it now occupies, enlarges considerably and becomes somewhat elongated.

A careful search has been made in the cytoplasm of the androcytes for accessory structures corresponding to the "chromatoider Nebenkörper" (IKENO 6) or "limosphere" (WILSON 10), the "percnosome" and the "apical body" (ALLEN 2) described by other investigators of bryophyte spermatogenesis; but, as WOODBURN (12) also reports, nothing which can confidently be regarded as such a body has been found. Occasionally there is observed in the cytoplasm a darker area, which, although it is as a rule rather vague in outline (fig. 7, below and at left of nucleus in each cell), may in certain cases be more definitely delimited (fig. 9). A similar appearance is also often seen in the later stages of spermatogenesis (figs. 15, 16, 18, 19). It may well be that we are dealing here with a limosphere or other accessory body, but without more trustworthy evidence for its constant presence and regularity in behavior, at present it does not seem advisable to attribute to this body any special significance in the case of *Blasia*. The cytoplasm of the androcyte frequently contains a large vacuole, which may or may not lie near the blepharoplast (fig. 8).

The blepharoplast now begins to undergo a series of transformations which ultimately result in the formation of the cilia-bearing thread of the spermatozoid. After elongating very slightly, as previously noted, the blepharoplast becomes constricted

(fig. 10, upper cell) and divides by a process of simple fission into two portions (fig. 10, lower cell). These two portions, or blepharoplast granules as they may be termed, often lie very close together, but in many cases they are so far apart that there can be no doubt that the fission is complete. As a rule one of the granules at once begins to elongate, while the other remains relatively unchanged, so that many cells show two bodies, one of them round and the other comma-shaped, lying close together near the cell membrane (fig. 11). At about this stage the granules usually move closer to the nucleus. The comma-shaped granule continues to elongate (fig. 12) and divides again; whether the other granule also divides or not is a difficult matter to determine. The granules continue to multiply by fission (fig. 13) until several are present in a row (figs. 14, 15); seven was the largest number counted with certainty. The granules now appear less distinct from one another; it seems that they gradually undergo a coalescence (figs. 14-16), but it may also be that some of the fissions are incomplete, some of the granules therefore never being entirely separate.

The nucleus at this time moves more closely against the beaded blepharoplast (fig. 15) and begins to draw out into a point by the side of the latter (fig. 16). Both nucleus and blepharoplast continue to elongate spirally, the association between them becoming constantly more intimate (fig. 17). Fig. 18 represents a cell like that of fig. 17 viewed from the direction indicated by the arrow; it is here seen that the blepharoplast is applied along one edge of the flattened point of the nucleus. As the transformation continues the boundary between nucleus and blepharoplast gradually becomes indistinguishable (fig. 19). Even at this late stage the irregular outline of the blepharoplast is still evident; the blepharoplast granules have not yet become so completely coalesced that the thread which they form is smooth in outline. The nucleus continues to elongate and condense, becoming increasingly slender, while two cilia grow out from the blepharoplast, which projects beyond the nucleus at the anterior end. The spermatozoid is now mature (fig. 20) and ready to escape from the antheridium.

### Discussion

The two main points wherein this description disagrees with that of WOODBURN (12) are as follows. First, according to that author there are no indications of centrosomes in the spermatogenous mitoses, the blepharoplast first appearing as a cytoplasmic differentiation in the androcyte. On the contrary, the present writer finds that centrosomes are present at all stages of the last mitosis, and that these persist as the blepharoplasts of the androcytes. Second, WOODBURN states that the blepharoplast in the androcyte undergoes a simple elongation to form the cilia-bearing thread, whereas the present writer sees it fragmenting to several pieces which coalesce to form the thread somewhat after the manner of the blepharoplasts of *Equisetum* and *Marsilia* (SHARP 8, 9).

It is not improbable that this disagreement is due in part to actual differences in the two lots of material studied. Although the single species of the genus, *Blasia pusilla*, was used in both instances, a comparison will show that the cells described in the present account are little more than half the size of those figured by WOODBURN. Although it is possible, therefore, that the two lots of material represent two varieties, too much weight should not be placed upon a size difference, for it is known in certain cases (*Equisetum*, SHARP 8) that androcytes and spermatozoids often vary considerably in size in the same lot of material.

Lack of agreement as to the presence of centrosomes during mitosis is perhaps not surprising. Because of their extreme minuteness the centrosomes might easily be overlooked in the stages previous to that at which WOODBURN first finds them, and at which they enlarge and become really conspicuous for the first time. With regard to the fragmentation of the blepharoplast, on the other hand, it is more difficult to understand why material actually the same should be interpreted so differently. In the writer's material the process of fragmentation is shown with great clearness; only occasionally is anything found in good preparations which might be interpreted as a uniformly elongating blepharoplast. Moreover, in no case has a condition approaching that shown in WOODBURN'S fig. 11 been observed. The nucleus becomes closely applied to the blepharoplast when the latter is in

the form of a short lumpy rod or series of granules, and at no time does the blepharoplast have the form of a long slender thread free from the nucleus as in WOODBURN'S figure. The writer, therefore, is inclined to attribute the disagreement for the most part to actual differences in the material studied rather than to differences in interpretation.

The phenomenon of fragmentation is probably the most interesting feature of the blepharoplast of *Blasia*. In all previous accounts of bryophyte spermatogenesis, including those of IKENO (6) on *Marchantia*, WILSON (10) on *Pellia*, *Polytrichum*, and *Atrichum*, WOODBURN (11, 12, 13) on several liverworts and *Mnium*, Miss BLACK (3) on *Riccia*, and ALLEN (2) on *Polytrichum*, the blepharoplast is reported to elongate without breaking up into smaller portions. ALLEN (2) states that "while the possibility of a somewhat similar occurrence [fragmentation] is suggested by the rather knotty appearance of the blepharoplast of *Polytrichum* when it begins to elongate, there is no time when it is visibly resolved into smaller bodies." In *Blasia*, therefore, we have the only known instance in bryophytes of such a fragmentation of the blepharoplast as occurs in *Equisetum*, *Marsilia*, and the cycads.

Although fragmentation is in general a characteristic of the blepharoplasts of the cycads, and only occasionally found in pteridophytes (*Equisetum* and *Marsilia*), it is now evident that it may occur in forms lower in the scale. Moreover, it is seen that it is not, as might be supposed, merely a means by which large blepharoplasts become transformed, for the blepharoplasts of *Equisetum* and *Marsilia*, and especially those of *Blasia*, are very small. Although the details of the process of fragmentation differ in the various cases (by simple fission in *Blasia* and by vacuolization in the other forms), it is scarcely to be doubted that the phenomenon is a result of similar causes in all. In attempting to find a possible historical reason for it, one is struck by the resemblance between the fission of the blepharoplast in *Blasia* (fig. 10) and the division of an ordinary centrosome before mitosis. If the blepharoplast actually represents a centrosome, as the writer (8) believes the evidence indicates, it is at least possible that its frequent fragmentation, in spite of the fact that in the more advanced

forms (cycads) this fragmentation becomes a very much modified process, may be a manifestation of the power of division which is one of the chief characteristics of centrosomes. According to this interpretation the first fission of the blepharoplast of *Blasia* (fig. 10) would correspond to the centrosome division which would normally occur if another mitosis were to take place, and the further fragmentation would represent a further manifestation of the centrosome's power of division which may have been retained from a time when more spermatozooids were produced from a mother cell, and which has in some way become a feature of the development of the cilia-bearing structures. In this way *Blasia* may shed light upon the origin of the remarkable behavior of the cycad blepharoplasts.

To this idea, which presents itself as a suggestion and may scarcely deserve to be proposed as a theory, there are obviously many objections. Chief among these is the fact that fragmentation is most conspicuous in the blepharoplasts of the cycads, but developed almost not at all in those of the bryophytes, which would be expected to have retained in the manner of their elongation more evidences of a derivation from normal centrosome division. It is possible, however, that the simple fission of the blepharoplast as seen in *Blasia*, was soon replaced in most bryophytes and pteridophytes by uniform elongation without fragmentation through the failure of the fission to occur after the slight elongation normally preceding it (figs. 7-9), this elongation then continuing to form the uniform cilia-bearing thread. Fragmentation would thus be a retained feature in *Blasia*, *Equisetum*, *Marsilia*, and the cycads, although the manner in which it is accomplished in the higher forms (through a complex process of vacuolization rather than simple fission) would still be regarded as an advanced feature subsequently evolved. Whether, therefore, the objection stated rules out the suggested explanation or not can scarcely be decided in view of the fact that the evidence at hand has been obtained from so few bryophytes and pteridophytes, comparatively speaking, and especially in view of our lack of adequate knowledge of blepharoplast origin and behavior in the algae.

A further objection may be seen in the case of animal spermatogenesis, in which an undoubted centrosome elongates without fragmentation as it performs its rôle in the development of the motor structures. It is noteworthy, however, that cilia are frequently seen growing from recently divided centrosomes in the case of certain insect spermatocytes (HENNEGUY 5) in much the same fashion that the cilia start to grow from the recently formed blepharoplast granules in *Equisetum* (SHARP 8). Moreover, in the Flagellata, which should furnish evidence more valuable than that in the higher animals, it is known that in certain cases blepharoplasts arise from functional centrosomes by division (see MINCHIN 7, pp. 82 ff.).

Although there is thus seen to be considerable evidence for the derivation of blepharoplast fragmentation from normal centrosome division, this evidence is probably best regarded as scarcely sufficient to warrant the establishment of such an interpretation as a general theory.

The question of the relation of the centrosome to the blepharoplast has been fully discussed by the writer in his papers on *Equisetum* and *Marsilia* (8, 9). It will be sufficient here to recall that the conclusions were reached that the blepharoplasts of bryophytes, pteridophytes, and gymnosperms are "ontogenetically or phylogenetically centrosomes" (IKENO); that these centrosomes become more and more restricted in the life history in passing upward through these groups; that they are retained in spermatogenous cells because of the biological importance of the cilia-bearing function which they there perform; and that in connection with this function they have become profoundly modified, losing many of the characteristics of centrosomes and assuming new characteristics not exhibited by centrosomes elsewhere.

To these conclusions *Blasia* furnishes support of no new kind; it merely confirms them by affording another example of blepharoplasts arising from centrosomes functional in mitosis. How extensive this centrosome behavior is in the case of *Blasia* the present study may not show, for, as stated in the description, the writer's material does not enable him to say whether the bodies in question arise from preexisting ones by division or not, or whether they



are present at only one or more than one spermatogenous mitosis. So far as actual evidence goes, it is possible to state unreservedly only that they are present from the stage represented in fig. 1 onward, and that through the single mitosis they appear to perform the usual functions of centrosomes. The discovery of fragmentation in the blepharoplast of a bryophyte serves to confirm the view that the blepharoplasts of all groups above the algae are homologous structures, and the details of the process aid materially in accounting for the behavior of those blepharoplasts which have become least centrosome-like.

### Summary

1. Centrosomes are present in *Blasia* at all stages of the mitosis which differentiates the androcytes, and in the androcytes they persist and function as the blepharoplasts.

2. In the transformation of the androcyte into the spermatozoid, the blepharoplast fragments repeatedly by simple fission, forming a number of distinct granules which coalesce to form a short lumpy rod. This rod elongates and becomes a more uniform thread bearing two cilia, while the nucleus also elongates in intimate union with it to form the body of the spermatozoid. The present instance is the first in which blepharoplast fragmentation has been reported in a bryophyte.

3. It is possible that the fission of the *Blasia* blepharoplast, and therefore the more complex fragmentation of the blepharoplasts of *Equisetum*, *Marsilia*, and the cycads, may be homologized with the normal division exhibited by ordinary centrosomes.

CORNELL UNIVERSITY

### LITERATURE CITED

1. ALLEN, C. E., Cell structure, growth, and division in the antheridia of *Polytrichum juniperinum* Willd. *Archiv Zellforschung* 8:121-188. pls. 6-9. 1912.
2. ———, The spermatogenesis of *Polytrichum juniperinum*. *Ann. Botany* 31:269-292. pls. 15, 16. 1917.
3. BLACK, CAROLINE A., The morphology of *Riccia Frostii* Aust. *Ann. Botany* 27:511-532. pls. 37, 38. 1913.

4. CHAMBERLAIN, C. J., Spermatogenesis in *Dioon edule*. BOT. GAZ. 47: 215-236. pls. 16-18. 1909.
5. HENNEGUY, L. F., Sur les rapports des cils vibratiles avec les centrosomes. Arch. d'Anat. Micr. 1:481-496. figs. 5. 1898.
6. IKENO, S., Die Spermatogenese von *Marchantia polymorpha*. Beih. Bot. Centralbl. 15:65-88. pl. 3. 1903.
7. MINCHIN, E. A., An introduction to the study of the Protozoa. London. 1912.
8. SHARP, L. W., Spermatogenesis in *Equisetum*. BOT. GAZ. 54:89-119. pls. 7, 8. 1912.
9. ———, Spermatogenesis in *Marsilia*. BOT. GAZ. 58:419-431. pls. 33, 34. 1914.
10. WILSON, M., Spermatogenesis in the Bryophyta. Ann. Botany 25:415-457. pls. 37, 38. figs. 3. 1911.
11. WOODBURN, W. L., Spermatogenesis in certain Hepaticae. Ann. Botany 25:299-313. pl. 25. 1911.
12. ———, Spermatogenesis in *Blasia pusilla*. Ann. Botany 27:93-101. pl. 11. 1913.
13. ———, Spermatogenesis in *Mnium affine* var. *ciliaris* (Grev.) C. M. Ann. Botany 29:441-456. pl. 21. 1915.

#### EXPLANATION OF PLATE XV

All figures were drawn at the level of the table with the aid of an Abbé camera lucida from cells stained with iron alum-haematoxylin. Examination of the cells was made under a Zeiss 2 mm. apochromatic objective, N.A. 1.40, but because of its slightly greater magnifying power a Spencer 2 mm. achromatic objective was used with an 18 ocular for outlining the drawings. The figures, which have not been reduced in reproduction, show a magnification of 4200 diameters.

FIG. 1.—Androcyte mother cell (penultimate spermatogenous cell) with two centrosomes.

FIG. 2.—Prophase of last spermatogenous mitosis; centrosomes at poles of developing spindle.

FIG. 3.—Metaphase; centrosomes at spindle poles.

FIG. 4.—Late anaphase; centrosomes present.

FIG. 5.—Telophase; centrosomes near daughter nuclei.

FIG. 6.—Late telophase; each cell has one centrosome (blephoroplast).

FIG. 7.—Androcytes (spermatids) rounded off; blepharoplast slightly elongated in each; dark body near nucleus.

FIG. 8.—Pair of androcytes with vacuoles in cytoplasm.

FIG. 9.—Androcyte with dark body (limosphere?) in addition to blepharoplast.

FIG. 10.—Pair of androcytes: blepharoplast undergoing fission in upper cell; two blepharoplast granules resulted from fission in lower cell.

FIG. 11.—Pair of androcytes showing elongation of one blepharoplast granule.

FIG. 12.—Androcyte; slightly later stage.

FIG. 13.—Blepharoplast granules multiplying.

FIG. 14.—Later stage; granules somewhat coalesced.

FIG. 15.—Nucleus moving against blepharoplast.

FIG. 16.—Nucleus elongating by side of blepharoplast; blepharoplast granules becoming coalesced.

FIG. 17.—Later stage; blepharoplast and nucleus becoming closely associated.

FIG. 18.—Cell like that of fig. 17 viewed from direction indicated by arrow; blepharoplast lying along edge of flattened point of nucleus.

FIG. 19.—Later stage; blepharoplast still irregular in outline; boundary between nucleus and blepharoplast indistinguishable.

FIG. 20.—Mature spermatozoid ready to escape from antheridium.